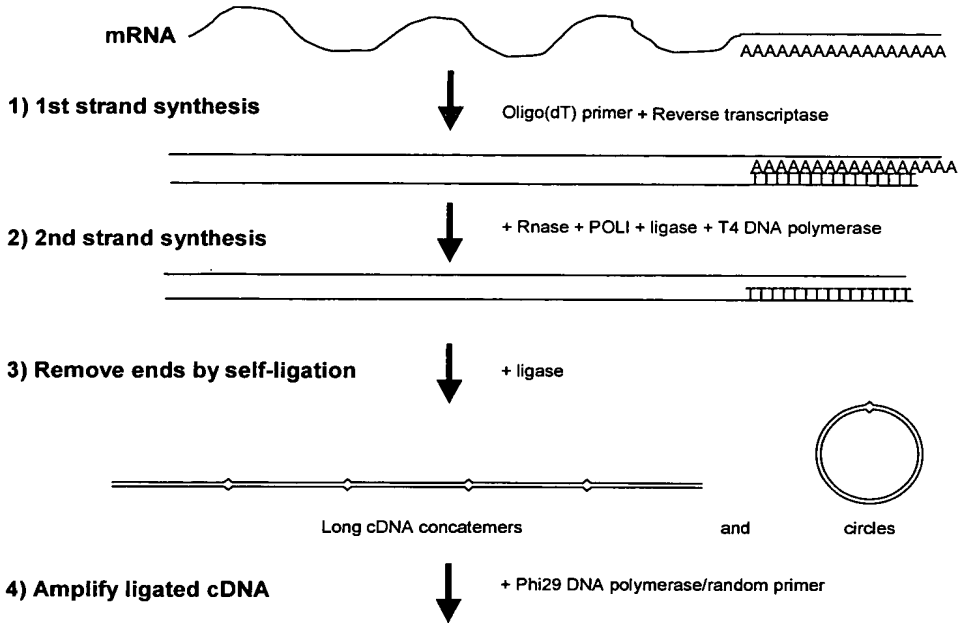

mRNA
AAAAAAAAAAAAAAAAA
1) 1st strand synthesis
Oligo(dT) primer + Reverse transcriptase
AAAAAAAAAAAAAAAAA
2) 2nd strand synthesis
+ Rnase + POLI + ligase + T4 DNA polymerase
3) Remove ends by self-ligation
+ ligase
Long cDNA concatemers
and
circles
4) Amplify ligated cDNA
+ Phi29 DNA polymerase/random primer

FIGURE 1

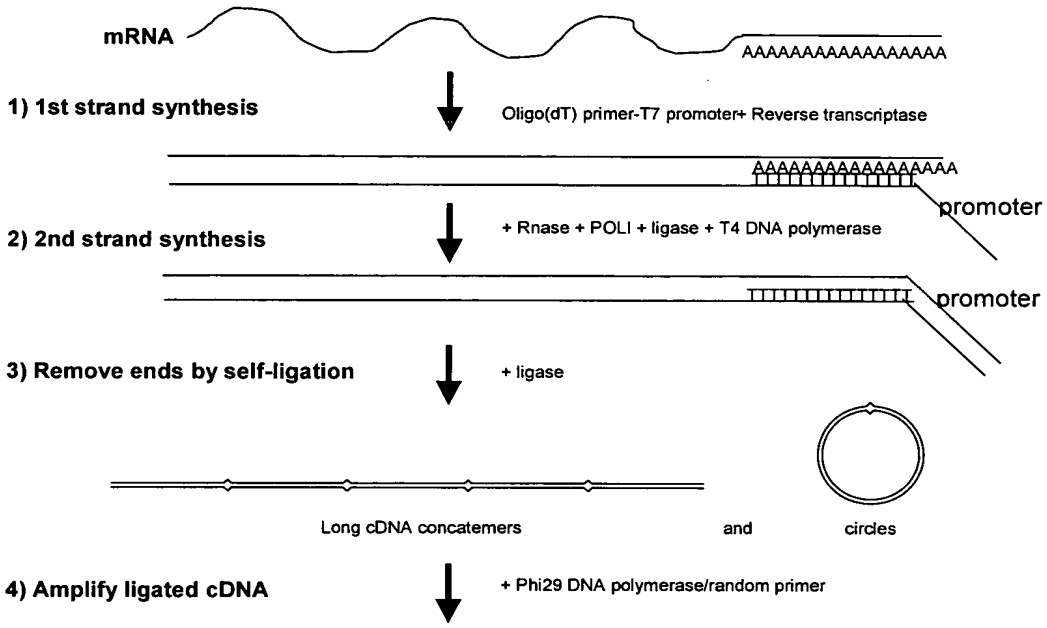

mRNA
AAAAAAAAAAAAAAAAA
1) 1st strand synthesis
Oligo(dT) primer-T7 promoter+ Reverse transcriptase
AAAAAAAAAAAAAAAAA
promoter
2) 2nd strand synthesis
+ Rnase + POLI + ligase + T4 DNA polymerase
promoter
3) Remove ends by self-ligation
+ ligase
Long cDNA concatemers
and
circles
4) Amplify ligated cDNA
+ Phi29 DNA polymerase/random primer

FIGURE 2

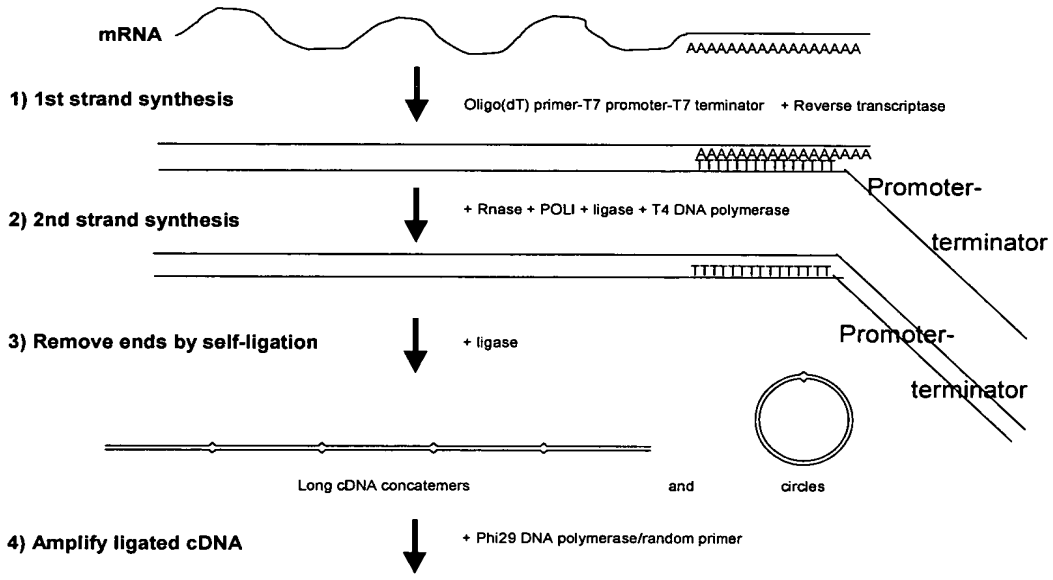

mRNA
AAAAAAAAAAAAAAAAA
1) 1st strand synthesis
Oligo(dT) primer-T7 promoter-T7 terminator + Reverse transcriptase
AAAAAAAAAAAAAAAAA
TTTTTTTTTTTTTTTTT
Promoter-
terminator
2) 2nd strand synthesis
+ Rnase + POLI + ligase + T4 DNA polymerase
TTTTTTTTTTTTTTTTT
Promoter-
terminator
3) Remove ends by self-ligation
+ ligase
Long cDNA concatemers
and
circles
4) Amplify ligated cDNA
+ Phi29 DNA polymerase/random primer

FIGURE 3

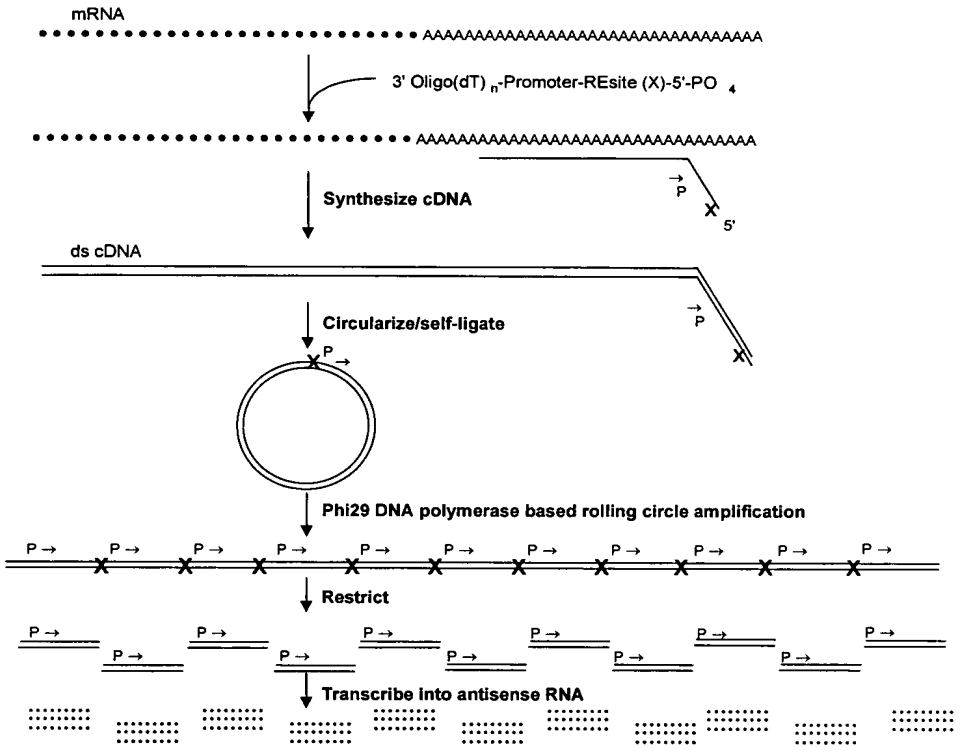

mRNA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
3' Oligo(dT) n-Promoter-REsite (X)-5'-PO 4
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
Synthesize cDNA
P
5'
ds cDNA
Circularize/self-ligate
P
Phi29 DNA polymerase based rolling circle amplification
Restrict
Transcribe into antisense RNA

FIGURE 4

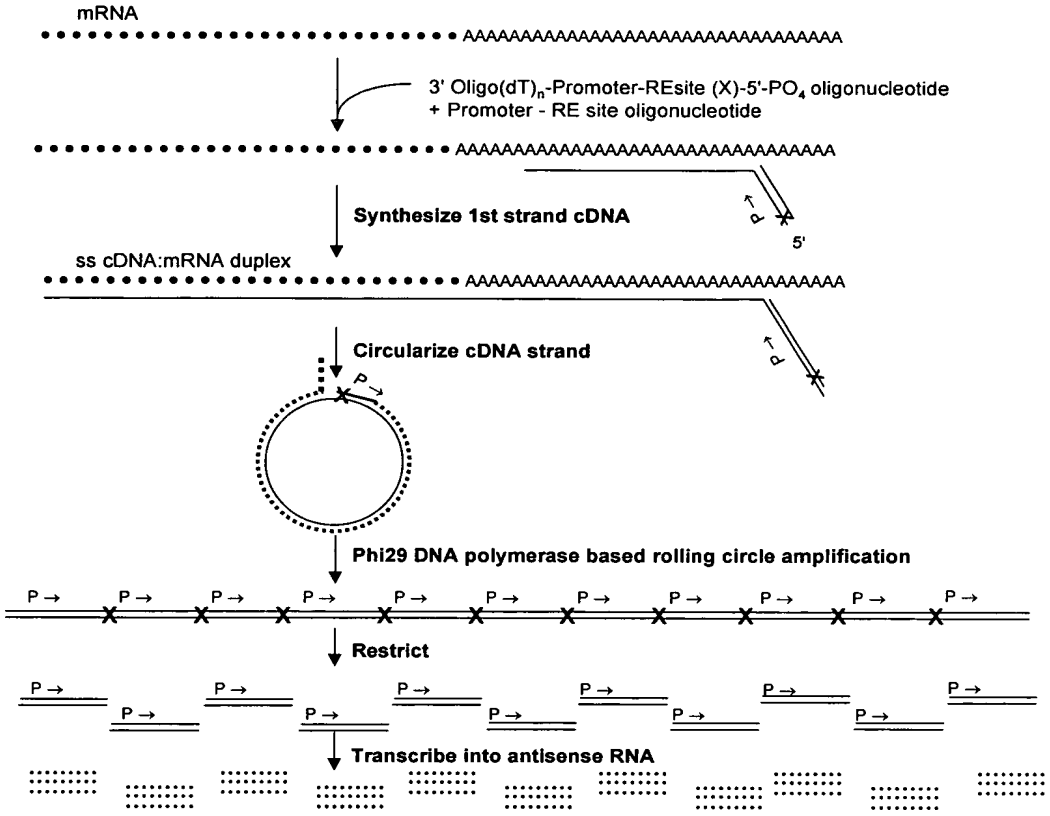
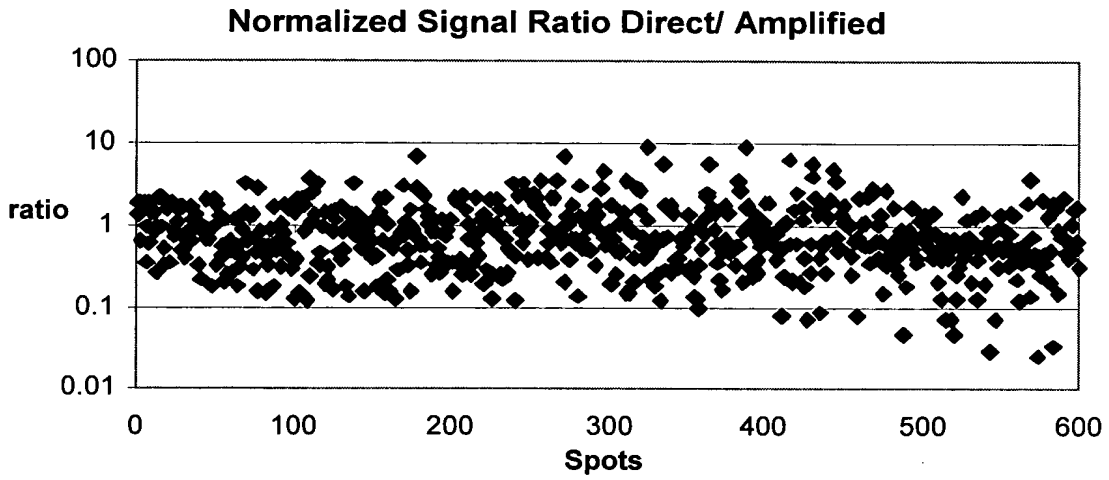
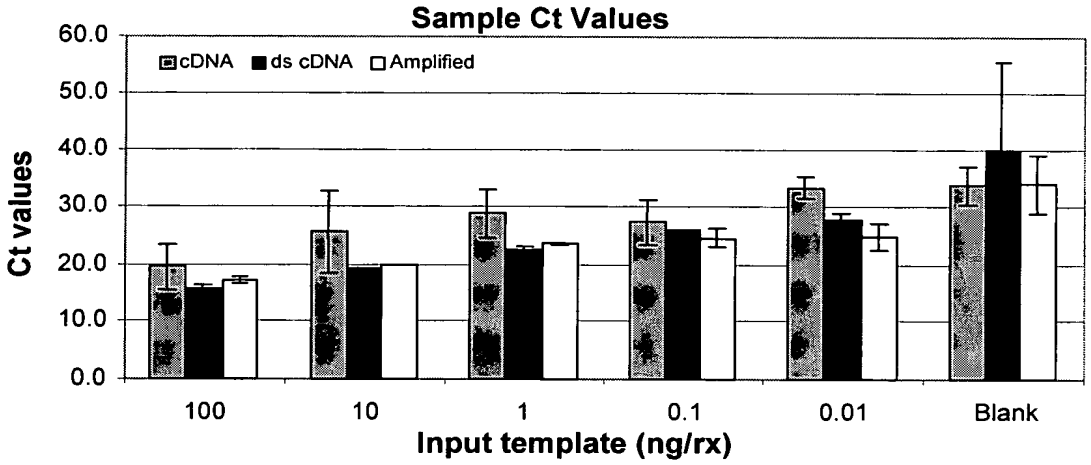

mRNA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
3' Oligo(dT)n-Promoter-REsite (X)-5'-PO4 oligonucleotide
+ Promoter - RE site oligonucleotide
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
Synthesize 1st strand cDNA
P
5'
ss cDNA:mRNA duplex
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
Circularize cDNA strand
P
Phi29 DNA polymerase based rolling circle amplification
P →
Restrict
Transcribe into antisense RNA

FIGURE 5



Comparison of expression levels using signal strengths obtained using labeled material from Skeletal Muscle tissue with a CyScribe first strand cDNA kit (Amersham Biosciences) or labeled material obtained after cDNA synthesis, circularization, amplification, restriction digest and in vitro transcription (as described). The plot shows the normalized signal ratios for 600 spots. These data indicate that gene expression information is accurately maintained using the described amplification procedure.

FIGURE 6



Beta actin gene specific primers were used to perform real-time quantitative PCR from either 1st strand cDNA, double stranded cDNA or double stranded cDNA which has been circularized and then amplified using random primers and Phi29 DNA polymerase based rolling circle amplification as described. Ct values were determined for each sample in a serial 10x dilution series. The similar Ct values for each amount of input nucleic acid sample suggests that the beta actin transcript concentration relative to the entire sample is accurately maintained throughout the procedure.

FIGURE 7